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(54) Title: USE OF EXENDINS AND AGONISTS THEREOF FOR THE REDUCTION OF FOOD INTAKE

(57) Abstract

Methods for treating conditions or disorders which can be alleviated by reducing food intake are disclosed which comprise administration of an effective amount of an exendin or an exendin agonist, alone or in conjunction with other compounds or compositions that effect satiety. The methods are useful for treating conditions or disorders, including obesity, Type II diabetes, eating disorders, and insulin-resistance syndrome. The methods are also useful for lowering the plasma glucose level, lowering the plasma lipid level, reducing the cardiac risk, reducing the appetite, and reducing the weight of subjects. Pharmaceutical compositions for use in the methods of the invention are also disclosed.

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USE OF EXENDINS AND AGONISTS THEREOF FOR THE REDUCTION OF FOOD INTAKE

This application claims the benefit of U.S. Provisional Application No. 60/034,905, filed January 7, 1997, U.S. Provisional Application No. 60/055,404, filed August 8, 1997, U.S. Provisional Application No. 60/066,029 filed November 14, 1997, and U.S. Provisional Application No. 60/065,442, November 14, 1997.

FIELD OF THE INVENTION

The present invention relates to methods for treating conditions or disorders which can be alleviated by reducing food intake comprising administration of an effective amount of an exendin or an exendin agonist alone or in conjunction with other compounds or compositions that affect satiety such as a leptin or an amylin agonist. The methods are useful for treating conditions or disorders, in which the reduction of food intake is of value, including obesity, Type II diabetes, eating disorders, and insulin-resistance syndrome. The methods are also useful for lowering the plasma lipid level, reducing the cardiac risk, reducing the appetite, and reducing the weight of subjects. Pharmaceutical compositions for use in the methods of the

containing such amino acids, may be prepared using methods known in the art. See, e.g., Bartlett and Landen, Biorg. Chem. 14:356-377 (1986).

The compounds described above are useful in view of their pharmacological properties. In particular, the compounds of the invention possess activity as agents to reduce food intake. They can be used to treat conditions or diseases which can be alleviated by reducing food intake.

Compositions useful in the invention may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular and subcutaneous) or nasal or oral administration. In some cases, it will be convenient to provide an exendin or exendin agonist and another food-intake-reducing, plasma glucose-lowering or plasma lipid-lowering agent, such as amylin, an amylin agonist, a CCK, or a leptin, in a single composition or solution for administration together. In other cases, it may be more advantageous to administer the additional agent separately from said exendin or exendin agonist. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral

Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988).

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. They can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 3.0 to 8.0, preferably at a pH of about 3.5 to 5.0. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other

inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

The claimed compositions can also be formulated as pharmaceutically acceptable salts (*e.g.*, acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic

acid, cyclohexylsulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, transmucosally, or by pulmonary inhalation.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methyl cellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic

surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

For use by the physician, the compositions will be provided in dosage unit form containing an amount of an exendin or exendin agonist, for example, exendin-3, and/or exendin-4, with or without another food intake-reducing, plasma glucose-lowering or plasma lipid-lowering agent. Therapeutically effective amounts of an exendin or exendin agonist for use in reducing food intake are those that suppress appetite at a desired level. As will be recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient's physical condition, the blood sugar level and other factors.

The effective daily appetite-suppressing dose of the compounds will typically be in the range of about 10 to 30

μg to about 5 mg/day, preferably about 10 to 30 μg to about 2 mg/day and more preferably about 10 to 100 μg to about 1 mg/day, most preferably about 30 μg to about 500 μg/day, for a 70 kg patient, administered in a single or divided doses. The exact dose to be administered is determined by the attending clinician and is dependent upon where the particular compound lies within the above quoted range, as well as upon the age, weight and condition of the individual. Administration should begin whenever the suppression of food intake, or weight lowering is desired, for example, at the first sign of symptoms or shortly after diagnosis of obesity, diabetes mellitus, or insulin-resistance syndrome. Administration may be by injection, preferably subcutaneous or intramuscular. Orally active compounds may be taken orally, however dosages should be increased 5-10 fold.

The optimal formulation and mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human subjects they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLE 1: Exendin Injections Reduced the Food Intake of Normal Mice

All mice (NIH:Swiss mice) were housed in a stable environment of 22 (± 2)° C, 60 (±10) % humidity and a 12:12 light:dark cycle; with lights on at 0600. Mice were housed in groups of four in standard cages with *ad libitum* access to food (Teklad: LM 485; Madison, WI) and water except as noted, for at least two weeks before the experiments.

All experiments were conducted between the hours of 0700 and 0900. The mice were food deprived (food removed at 1600 hr from all animals on day prior to experiment) and individually housed. All mice received an intraperitoneal injection (5 μl/kg) of either saline or exendin-4 at doses of 0.1, 1.0, 10 and 100 μg/kg and were immediately presented with a pre-weighed food pellet (Teklad LM 485). The food pellet was weighed at 30-minute, 1-hr, 2-hr and 6-hr

intervals to determine the amount of food eaten.

Figure 1 depicts cumulative food intake over periods of 0.5, 1, 2 and 6hr in overnight-fasted normal NIH:Swiss mice following ip injection of saline, 2 doses of GLP-1, or 4 doses of exendin-4. At doses up to 100μg/kg, GLP-1 had no effect on food intake measured over any period, a result consistent with that previously reported (Bhavsar, S.P., et al., Soc. Neurosci. Abstr. 21:460 (188.8) (1995); and Turton, M.D., Nature, 379:69-72, (1996)).

In contrast, exendin-4 injections potently and dose-dependently inhibited food intake. The ED_{50} for inhibition of food intake over 30 min was 1μg/kg, which is a level about as potent as amylin (ED_{50} 3.6μg/kg) or the prototypical peripheral satiety agent, CCK (ED_{50} 0.97μg/kg) as measured in this preparation. However, in contrast to the effects of amylin or CCK, which abate after 1-2 hours, the inhibition of food intake with exendin-4 was still present after at least 6 hours after injection.

EXAMPLE 2: Exendin Reduced the Food Intake of Obese Mice

All mice (female ob/ob mice) were housed in a stable environment of 22 (±2)° C, 60 (±10) % humidity and a 12:12 light:dark cycle; with lights on at 0600. Mice were housed in groups of four in standard cages with *ad libitum* access to food (Teklad: LM 485) and water except as noted, for at

least two weeks before the experiments.

All experiments were conducted between the hours of 0700 and 0900. The mice were food deprived (food removed at 1600 hr from all animals on day prior to experiment) and individually housed. All mice received an intraperitoneal injection (5 μl/kg) of either saline or exendin-4 at doses of 0.1, 1.0 and 10 μg/kg (female ob/ob mice) and were immediately presented with a pre-weighed food pellet (Teklad LM 485). The food pellet was weighed at 30-minute, 1 -hr, 2-hr and 6-hr intervals to determine the amount of food eaten.

Figure 2 depicts the effect of exendin-4 in the ob/ob mouse model of obesity. The obese mice had a similar food intake-related response to exendin as the normal mice. Moreover, the obese mice were not hypersensitive to exendin, as has been observed with amylin and leptin (Young, A.A., et al., Program and Abstracts, 10th International Congress of Endocrinology, June 12-15, 1996 San Francisco, pg 419 (P2-58)).

EXAMPLE 3: Intracerebroventricular Injections of Exendin Inhibited Food Intake in Rats

All rats (Harlan Sprague-Dawley) were housed in a stable environment of 22 (±2)° C, 60 (±10)% humidity and a 12:12 light:dark cycle; with lights on at 0600. Rats were